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High prevalence of community-acquired norovirus gastroenteritis among hospitalized children: a prospective study

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Abstract

Acute gastroenteritis (AGE) causes significant morbidity, especially in young children, and frequently requires hospitalization even in developed countries. Surveillance studies of AGE are important to determine the prevalence and variety of bacterial and viral pathogens, to initiate targeted preventive measures, such as vaccine programmes, and to monitor its impact. A prospective study was conducted in children <5 years old, admitted with AGE between April 2006 and April 2007 to the Virgen del Rocío University Hospital, Seville, Spain. Demographic and clinical data were collected and patients followed-up after hospital discharge. A stool sample from each child was screened for enteropathogenic bacteria and tested by reverse transcription polymerase chain reaction for rotavirus, astrovirus, norovirus and sapovirus and by the immunochromatographic method for enteric adenoviruses. Norovirus was the most common pathogen in hospitalized children, being detected in 27%, followed by rotavirus 21%. Mixed infection occurred in nearly 20% of all norovirus infections and was most commonly associated with Salmonella spp. Rotavirus infection was associated with an overall higher severe clinical score compared with norovirus infection. Lactose intolerance was observed in 29 children (7.5%) and most commonly due to rotavirus infection ($p < 0.001$). Seizures were reported in four children. Norovirus was the commonest cause of AGE in hospitalized children <5 years during 2006–2007 in Seville, Spain. The use of these molecular techniques should be included routinely for the surveillance of sporadic cases and outbreaks of norovirus AGE in children attending hospitals as well as healthcare centres.

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Introduction

Acute gastroenteritis (AGE) is prevalent worldwide and associated with high mortality rates in developing countries; AGE causes significant morbidity, especially in young children, and frequently requires hospitalization even in developed countries [1]. Multiple bacterial and viral pathogens have been identified in the stools of children with gastroenteritis [2,3]. Rotaviruses are generally considered to be the major aetiological agents of severe infantile diarrhoea worldwide, and are responsible for over 500 000 deaths/year in children <5 years of age [4].

Although noroviruses were the first viruses to be clearly associated with AGE [5], their role as important pathogens in gastroenteritis outbreaks and acute sporadic enteric infection in children worldwide has been increasingly recognized since the introduction of the reverse transcription polymerase chain reaction (RT-PCR) method.

Recently, norovirus has been identified as the second most common cause of viral diarrhoea in children requiring treatment in hospital [6–8]. However, norovirus RT-PCR is

not generally used routinely in a clinical microbiology service, so the burden of this disease is likely to be underestimated and epidemiological studies are therefore hampered [9].

Studies from several European countries provide data on the incidence of AGE, risk factors, and the associated health burden. Findings are likely to differ within European countries due to environmental factors and differences in healthcare networks and nutritional habits [10–12].

Moreover, associated risk factors and complications are increasingly recognized, such as the association between viral AGE and seizures [13–15]. Continuous surveillance studies of AGE are important to determine the prevalence and variety of bacterial and viral pathogens, to initiate targeted preventive measures, such as vaccine programmes, and to monitor its impact [16,17]. The aim of this prospective study was to determine the impact of viral and bacterial pathogens on our paediatric population group suffering from AGE prior to the introduction of the rotavirus vaccine.

Materials and Methods

This study was carried out at the Virgen del Rocío University Hospital, a tertiary paediatric referral centre for Seville, serving a population of 123 225 children <5 years of age.

From 1 April 2006 through to 1 April 2007, children under 5 years of age with a community-acquired AGE were evaluated at healthcare centres and hospital accident and emergency (A + E) departments. The children attending the A + E department who were admitted to the hospital were prospectively enrolled in the study. Exclusion criteria were age ≥5 years, and diagnosis of an immunodeficiency or gastrointestinal tract disease.

AGE was defined as at least three looser than normal stools within a 24-h period or an episode of forceful vomiting with any loose stools lasting <14 days [6].

A stool sample from each child was collected within the first 3 days of the episode, transported immediately to the Microbiology Department and stored at 4°C until processing. All faecal samples were screened for enteropathogenic bacteria agents by conventional culture methods. Stool samples were screened for the presence of rotavirus (group A) and adenovirus antigens by EIA, biorapid ROTA-ADENO (Biokit, Barcelona, Spain) and sent to the reference laboratory (Viral Gastroenteritis Unit, National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain). Samples were processed as previously described and screened for rotavirus, astrovirus, norovirus, sapovirus and enteric adenoviruses [6]. Norovirus was only tested in samples from hospitalized children.

G and P rotavirus genotyping was performed in a total of 80 rotavirus strains using RT-PCR methods [18]. The 20-point Vesikari scoring system was used to summarize the clinical presentation. The scoring system includes items such as vomiting, presence of fever, and presence and severity of dehydration [19].

Complications were defined as the occurrence of extra-intestinal presentations of AGE. Clinical and demographic data were collected using a standardized case report form. Information about secondary lactose intolerance was obtained by telephone interviews within 1 month of discharge.

Statistical analysis

The SPSS version 15.0 statistical package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, the Mann–Whitney test or the Kruskal–Wallis test for continuous variables, and Pearson's chi-square test or Fisher's exact test, when appropriate, for categorical variables.

Results

During the 13-month study period, 2375 children <5 years of age were diagnosed with a community-acquired AGE. A total of 1845 cases were evaluated at healthcare centres whereas 530 children attended the hospital accident and emergency (A + E) department; 90% (n = 411) of the children attending the A + E department were admitted to the hospital, and 399 of these were included in this study.

Aetiology of sporadic cases of acute gastroenteritis

One specimen per patient was collected: 1845 from children who attended community healthcare centres, and 530 from children attending the A + E department (out of these, 399 samples were from subsequently hospitalized children).

TABLE 1. Aetiology of AGE in children attending healthcare centres (HCC) or being hospitalized (H)

Specimens (total)	HCC *n* (%) 1845 (100)	H *n* (%) 399 (100)	p
Norovirus*	n.a	107 (26.8)	n.a
Rotavirus	115 (6.2)	80 (20.0)	**<0.001**
Adenovirus	32 (1.7)	6 (1.5)	n.s
S. enteritidis	253 (13.7)	33 (8.3)	**<0.001**
C. jejuni	219 (11.9)	37 (9.3)	n.s
Yersinia spp	2 (0.1)	0	n.s
Others	146 (7.9)	2 (0.5)	**<0.001**
Total viral isolates	147 (7.9)	193 (48.3)	**<0.001**
Total bacterial isolates	474 (25.6)	70 (17.5)	**<0.001**
Total of isolated pathogens	767 (41.5)	265 (66.4)	**<0.001**

*This virus was not studied in outpatients. Others = due to bacterial overgrowth secondary to antibiotic therapy.
n.a., not applicable; n.s., not significant.

TABLE 2. Viral aetiology of AGE among hospitalized children

Pathogens	No. cases	% of total samples (399)
Norovirus	86	
Norovirus + *S. enteritidis*	14	
Norovirus + *C. jejuni*	3	
Norovirus + adenovirus	3	
Norovirus + rotavirus	1	
Total norovirus infections	107	26.8
Rotavirus	77	
Rotavirus + *S. enteritidis*	2	
Rotavirus + *C. jejuni*	1	
Total rotavirus infections	80	20
Adenovirus	2	
Adenovirus + *C. jejuni*	1	
Total adenovirus infections	6	1.5
Total virus infections	218	54.6

TABLE 3. Epidemiological and clinical characteristics of hospitalized children with AGE due to norovirus and rotavirus

Variable	No. of cases (%)		
	Norovirus, *n* = 86	Rotavirus, *n* = 77	p
Age in months (SD)	12 (1–56)	11 (1–52)	n.s
Male sex (%)	57 (66.2)	41 (53.2)	n.s
Nursery	5 (5.8)	17 (22)	**<0.001**
Breast-feeding	17 (19.7)	17 (22)	0.006
Vesikari severity score (>11)	13 (15.1)	33 (42.8)	**<0.001**
Vomiting	48 (55.8)	57 (74)	**<0.001**
Fever	68 (79)	63 (81.8)	0.002
Dehydration (1–5%)	18 (20.9)	45 (58.4)	**<0.001**
Intravenous rehydration	7 (8.1)	28 (36.3)	**<0.001**
Lactose intolerance	3 (3.4)	13 (16.8)	**<0.001**

n.s., not significant.

At least one pathogen was isolated in 41.5% (n = 767) of the children attending outpatient healthcare centres, compared with 66.4% (n = 265) of the hospitalized children (p <0.001, Table 1). Importantly, norovirus was only tested in samples from hospitalized children. Rotavirus and *Salmonella enteritidis* were significantly more prevalent in stools from inpatients, as summarized in Table 1.

Among hospitalized children, a viral aetiology was demonstrated in 54.6% (n = 218) of AGEs (Table 2). In these patients, norovirus was the most common pathogen, being detected in 26.8% (n = 107) of specimens, of which 21 cases had mixed infection (Table 2). Rotavirus accounted for 20% (n = 80; four cases with mixed infection). Adenovirus was present in 10 cases whereas astrovirus and sapovirus were not detected in any stool sample.

A bacterial pathogen was detected in 17.5% (n = 70) of the cases. *Campylobacter jejuni* was isolated from 37 specimens and *Salmonella enteritidis* from 33 specimens. This latter microorganism was most commonly found in co-infections with norovirus (n = 14).

RT-PCR for G and P rotavirus genotypes were performed on the 80 rotavirus-positive samples, being fully determined in 75% of the specimens. G1P[8] was the most prevalent association, being identified in over 50% of the samples, followed by G9P[8] (14%). Other associations were identified in ≤2 cases and included G2P[8], G3P[8] and G1P[4] (data not shown).

Demographic, epidemiological and clinical characteristics

Data on demographic, epidemiological and clinical characteristics of AGE cases among hospitalized children as well as relevant data obtained from the scoring Vesikari system were fully available in 97% (n = 386) of patients and are summarized in Table 3.

Rotavirus infection was associated with an overall higher severe clinical score compared with norovirus infection. Patients with rotavirus infections suffered significantly more from fever, vomiting and dehydration, requiring intravenous fluids. Day care attendance and breast feeding were also significantly associated with rotavirus infection, as the development of secondary lactose intolerance. Norovirus mixed infection presented with a higher clinical score compared with norovirus single infections; however, these findings were not significant (Vesikari score 10.5 vs. 12, p 0.6). Lactose intolerance was observed in 29 children (7.5%) and was most commonly due to rotavirus infection (p<0.001). Seizures were reported in four children (one each due to rotavirus, norovirus, *Campylobacter jejuni* and *Salmonella enteritidis*). There were no case fatalities. The seasonal distribution of norovirus detection showed two peaks of incidence, first in summer (64% of cases occurred from June through to August) and a second in winter (14% cases). However, the seasonality of norovirus detection was related to cases of mixed norovirus-*Salmonella enteritidis* infection, with a peak that was detected in July (13% cases).

Discussion

In this prospective study the epidemiology and clinical features of AGE in hospitalized children <5 years old are described. Norovirus was the most common pathogen detected (29%), followed by rotavirus (21%). In addition, norovirus occurred in a significant proportion of cases as mixed infections, and it was found to have a milder clinical course compared with rotavirus infections.

As the prevalence of norovirus infections in children attending healthcare centres was not determined, these children might account for an important proportion of two-thirds of cases with unknown aetiology in this study [20,21]. Over the last years, several studies have confirmed an important role for norovirus in sporadic cases of AGE worldwide [10,11,22].

A recent systematic literature review evaluated the role of norovirus in AGE using RT-PCR [5]. The prevalence of norovirus infection among paediatric outpatients with AGE ranged from 5.5% to 18.5%. In an Italian study, norovirus was isolated in 31% of samples from children with sporadic gastroenteritis who attended the A + E department [23]. Few data are published on AGE due to norovirus in Spanish children: norovirus was the second most frequent pathogen, being detected in 17.3% in a recent study conducted during a 3-year period among hospitalized children in a single centre [6]. In this study the worldwide-dominant GII.4 genotype was identified in 75% of norovirus-positive samples.

Rotavirus was the second most common cause of AGE in our study. The G1P[8] genotype was most prevalent in Seville as it was the case in other European countries over the last years [18]. There are no data available regarding circulating rotavirus genotypes in our geographical location. However, a major shift in genotype distribution has been observed over the last years in Spain: G4 strains were dominant between 1998 and 2000, followed by a gradual increase of the G1 genotype to 75% during 2000–2002. In 2005 the G9 strain, first detected in 1998, became the most prevalent genotype [24]. It is likely that vaccine pressure will further result in significant changes in predominant rotavirus genotypes in the forthcoming years, as has been reported in other parts of the world [22]. Surveillance of seasonal rotavirus serotype patterns is therefore of great importance in order to assess the impact of vaccines on circulating and emerging genotypes [25].

Further pathogens isolated from children with sporadic AGE were *C. jejuni* and *Salmonella* spp., followed by adenoviruses. *C. jejuni* is generally regarded as an important cause of diarrhoea in children <5 years of age. Indeed, in most developed countries, infection with *Campylobacter* species is associated with two age peaks (<5 and >65 years of age)[26]. Parasitic infections were not included in the diagnostic panel in our study as they are of minor importance as causes of community-acquired AGE in Spain [3].

Mixed infection occurred in nearly 20% of all norovirus infections and was most commonly associated with *Salmonella* spp. These observations support previous published data where norovirus mixed infections were reported in up to 24% of AGE cases attending the A + E department [17]. There exist only limited data from studies evaluating the clinical relevance of norovirus mixed infections. In two French studies viral mixed infections appeared not to be associated with a more severe clinical course compared with norovirus infection alone [15,27]. These results are in contrast to a Peruvian study where norovirus co-infections were associated with a higher clinical score [28]. Differences may be related to the co-infecting organism (viral co-infection in the French studies compared with bacterial co-infections in the Peruvian study). In our study no significant differences were observed; this might have been due to the low numbers of cases with co-infections.

Norovirus infection was shown to be clinically less severe than rotavirus AGE. This is supported by previously published data [6]. Lactose intolerance occurred significantly less during the recovery period for norovirus infection compared with rotavirus infection in hospitalixed children. Although this is regarded as a mild complication, it may cause prolonged symptoms, resulting in an increased number of healthcare follow-up visits. Benign seizures as a complication of viral AGE were also observed in this study, confirming previously published data [29].

Our study has several limitations. Firstly, the limited study period did not allow a long-term analysis of the epidemiology of AGE, which may change on a year-to-year basis. Secondly, the true significance of norovirus mixed infections in our population could not be clearly determined as the prevalence of norovirus was only determined in hospitalized children and not in asymptomatic carriers or symptomatic children attending the A + E department. Finally, the diagnosis of lactose intolerance relied on verbal information from parents with no laboratory confirmation. However, this study prospectively and extensively investigated AGE in a well-defined population, both microbiologically and clinically, including follow-up after hospital discharge.

In conclusion, this is the first study identifying norovirus as being the commonest cause of AGE in hospitalized children <5 years in Spain and further supporting its importance in the aetiology of AGE. Dual infections were common findings. The use of molecular techniques should be included routinely for the surveillance of sporadic cases vs. outbreaks of norovirus AGE in children attending hospitals as well as healthcare centres. This will further enable us to understand the epidemiology, the incidence and the burden of this disease and facilitate targeted preventive measures in the future.

Transparency Declaration

Conflicts of interest: nothing to declare.

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